STEPHEN AUSMUS (D245-9)

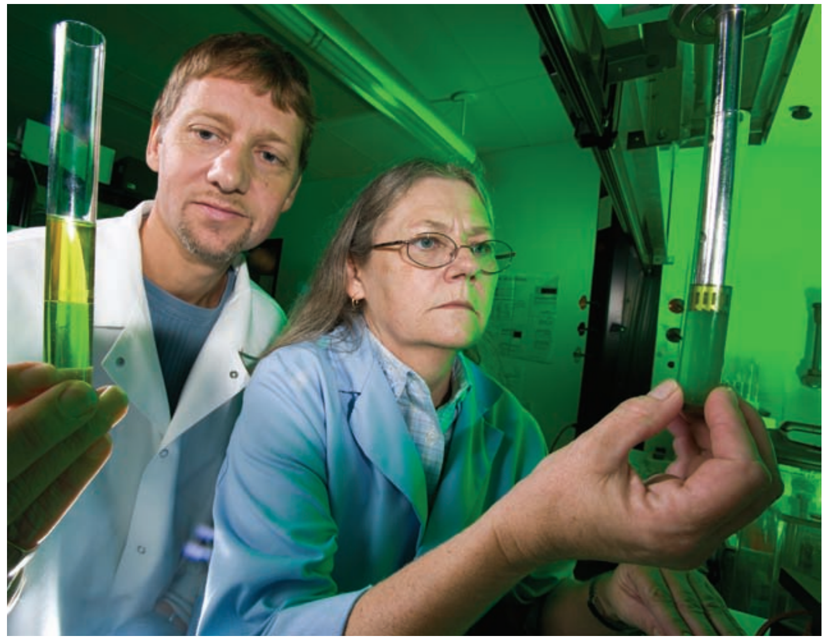
Pegging a Trouble-some Change in Hydrilla

Mutation seems to cause recently found resistance to a key herbicide.

The invasive plant known as hydrilla, *Hydrilla verticillata,* is sometimes called the perfect aquatic weed because of its reproductive adaptability. Since its introduction into the United States from Southeast Asia in the 1950s near Tampa, Florida, hydrilla has clogged waterways throughout the southeastern United States. It has even found its way to the nation's Pacific coast.

Mike Netherland (left) and Donald Morgan of the Army Corps of Engineers collect herbicide-resistant hydrilla from Lake Seminole in northern Florida.

STEPHEN AUSMUS (D247-2)



Technician J'Lynn Howell (right) homogenizes hydrilla samples while plant physiologist Franck Dayan observes the presence of carotenoids (the yellow layer in the test tube) that indicate the plant is herbicide resistant.

Now it appears that some hydrilla is becoming resistant to the herbicide that has been most effective against it.

Researchers in the ARS Natural Products Utilization Research Unit at Oxford, Mississippi, and SePRO Corporation, a firm based in Carmel, Indiana, that specializes in plant-protection management, have pegged this change to a gene mutation.

In fact, these scientists—plant physiologist Franck Dayan and molecular biologists Brian Scheffler, Albrecht Michel, and Renee Arias at Oxford, along with a SePRO team led by research director for aquatics Michael Netherland—submitted a patent application for the mutation's use. Michel and Arias are no longer with ARS, and Netherland is now with the U.S. Army Corps of Engineers.

How Hydrilla Adapts

Rooted in bottom sediments, hydrilla grows long, thin stems that rapidly reach the water's surface and form a thick mat. It spreads quickly through reproductive versatility and its ability to adapt to adverse conditions. "Infestations impair flood control, navigation, and recreation," says Dayan. "Also, dense growth can ruin water quality and displace valuable native aquatic plants."

The resistant hydrilla is a dioecious, female form that reproduces asexually. A monoecious form—that is, with both male and female flowers on the same plant—appeared in the mid-Atlantic states years after the dioecious biotype was found in Florida. It, to date, has not shown herbicide resistance.

For two decades, the herbicide fluridone has been a critical tool for fighting hydrilla selectively on a large scale. It is the only product approved by the U.S. Environmental Protection Agency for systemic control of the weed.

Fluridone acts by inhibiting the enzyme phytoene desaturase (PDS). As a result, phytoene—a colorless carotenoid—accumulates in leaves at the expense of protective, colored carotenoids. This process ultimately interferes with photosynthesis, thus killing the plant.

About 5 years ago, SePRO scientists noticed a drop in fluridone's efficacy in a few Florida waterways. Perplexed, they contacted ARS scientists at the Oxford laboratory and joined them in a cooperative research and development agreement aimed at determining why this drop was occurring.

"What we found surprised us," says Dayan. "The plant had selected a mutated form of the PDS-producing gene, resulting in a new, herbicide-resistant biotype. This was a huge discovery! Development of resistance to this herbicide had never been documented in an asexually reproducing higher plant—aquatic or terrestrial."

Steve Cockreham, vice president of research and regulatory affairs for SePRO, says that the tolerance has not been observed outside Florida.

More Fluridone, More Costs

"Historically, it has taken very low levels of fluridone to control hydrilla," says Scheffler, who now leads the ARS Mid-South Area Genomics Laboratory in Stoneville, Mississippi. But now, much higher concentrations of fluridone—though still within the recommended usage range—are needed to control resistant hydrilla.

"Since resistance means you need more herbicide, it also means higher treatment costs. And environmental impacts must also be considered."

The current research can help identify which hydrilla biotypes are in a lake. "This should help ensure proper application rates of fluridone for best control at minimal costs," Scheffler says.

Resistant biotypes were found in water that had been treated with fluridone for many years. Dayan reports that resistance in at least three hydrilla biotypes was two to five times that in susceptible hydrilla.

The researchers found that each of these biotypes had mutations in the PDS-producing gene. The mutations changed a key amino acid—arginine—into one of three other amino acids: histidine, cysteine, or serine. Dayan says all three mutations yielded herbicide-resistant PDS enzymes, but "the ecological fitness of the weed is apparently unaltered, enabling the resistant biotypes to become the dominant populations.

"We're still studying the precise reason for the resistance. But we do know that the mutation changes a weed that is susceptible to fluridone into one that resists it.

"The bottom line is that resistant hydrilla can spread quickly. Tools for selective control are very limited, and managing it has become much more costly and technically challenging."

Another First—Passing On the Resistance

Earlier, Scheffler and Dayan studied the PDS-producing gene in maize to be sure that the mutation did indeed cause resistance to fluridone. They introduced the mutation to the gene and produced an enzyme several times more resistant than the susceptible enzyme.

"This was the first documented instance of PDS genes from a plant conferring resistance to this class of herbicides," says Dayan.

In addition to submitting a patent application for use of the mutated gene, the researchers in Oxford and at SePRO are studying the hydrilla PDS gene in *Arabidopsis thaliana,* a flowering mustard plant that is also known as mouse-ear cress or thale cress. It is the workhorse of plant biotechnology because it has minimal genetic material and reproduces quickly.

"From this, we hope to learn more about cross-resistance to a variety of PDS inhibitors," says Dayan. "We also want to further evaluate this discovery's agronomic potential in other plant species."

He says early data from work with *Arabidopsis* points to the modified plant being "extremely resistant" to the herbicide.

Other research goals include assessing potential problems that could arise if this resistance were to occur in crops. Other PDS inhibitors, such as norflurazon, are used in soybean, cotton, and peanut fields to control many grasses, weeds, and sedges.

Meanwhile, concerns over the resistant hydrilla persist. Researchers fear potential cross-pollination between monoecious and dioecious hydrilla in areas where both biotypes grow together. That could result in hydrilla with even more genetic variability and greater adaptability to the environment.

Seeking Effective New Strategies

"What makes the situation serious is the current lack of alternatives," says Dayan. "No other aquatic herbicide can be used on as large a scale as fluridone."

Biological agents are being studied for controlling hydrilla, including some insects and the fungal pathogen *Mycoleptodiscus terrestris,* which induces a brief disease in the weed. SePRO has worked with the ARS National Center for Agricultural Utilization Research in Peoria, Illinois, to develop this agent. When used along with fluridone, the fungus seems to increase hydrilla's susceptibility to the herbicide.

Other removal methods include dredging as well as introducing triploid grass carp, *Ctenopharyngodon idella*. This herbivorous fish can control hydrilla, but it often eats most of the native vegetation, too.

STEPHEN AUSMUS (D246-1)

Franck Dayan and support scientist Susan Watson use high-performance liquid chromatography to measure production of carotenoids by hydrilla.

"Ultimately, hydrilla's development of herbicide resistance has led industry, state, and federal agencies to team up to develop new strategies to fight the aquatic weed," says Dayan. These include monitoring lakes regularly to keep the spread of resistant hydrilla in check.

"There's no doubt that water-resource managers in affected regions may be in for long-term ecological and economic problems," Dayan adds. "But the scientific community hopes to help them better manage hydrilla through new studies and by using what we know about fluridone resistance to our benefit."—By **Luis Pons,** ARS.

This research is part of Water Quality and Management (#201) and Plant Biological and Molecular Processes (#302), two ARS National Programs described on the World Wide Web at www.nps.ars.usda.gov.

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